TITLE: Mendelian randomization of circulating polyunsaturated fatty acids and colorectal cancer risk

AUTHORS: Nikhil K. Khankari [1], Barbara L. Banbury [2], Maria C. Borges [3,4], Philip C. Haycock [3,4], Demetrius Albanes [5], Volker Arndt [6], Sonja I. Berndt [5], Stéphane Bézieau [7], Hermann Brenner [6,8,9], Peter T. Campbell [10], Graham Casey [11], Andrew T. Chan [12–17], Jenny Chang-Claude [18,19], David V. Conti [20], Michelle Cotterchio [21], Dallas R. English [22,23], Jane C. Figueiredo [20,24], Graham G. Giles [22,23], Edward L. Giovannucci [13,16,25], Marc J. Gunter [26], Jochen Hampe [27], Michael Hoffmeister [6], John L. Hopper [22], Mark A. Jenkins [22], Amit D. Joshi [14,16], Loic Le Marchand [28], Mathieu Lemire [29], Christopher I. Li [2], Li Li [30], Annika Lindblom [31,32], Vicente Martín [33], Victor Moreno [34–36], Polly A. Newcomb [2,37], Kenneth Offit [38,39], Paul D. P. Pharoah [40], Gad Rennert [41–43], Lori C. Sakoda [2,44], Clemens Schafmayer [45], Stephanie L. Schmit [20,46], Martha L. Slattery [47], Mingyang Song [12,14,16,25], Stephen N. Thibodeau [48], Cornelia M. Ulrich [49], Stephanie J. Weinstein [5], Emily White [2,37], Aung Ko Win [22], Alicja Wolk [50], Michael O. Woods [51], Anna H. Wu [20], Qiuyin Cai [1], Joshua C. Denny [52,53], Todd L. Edwards [1,52], Harvey J. Murff [1], Stephen B. Gruber [54], Ulrike Peters [2,37], Wei Zheng [1]

AFFILIATIONS:

1. Division of Epidemiology, Department of Medicine, Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, Tennessee, USA.
2. Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA.
3. Medical Research Council (MRC) Integrative Epidemiology Unit, University of Bristol, Bristol, UK.
4. Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK.
5. Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA.
6. Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ), Heidelberg, Germany.

7. Service de Génétique Médicale, Centre Hospitalier Universitaire (CHU) Nantes, Nantes, France.

8. Division of Preventive Oncology, German Cancer Research Center (DKFZ) and National Center for Tumor Diseases (NCT), Heidelberg, Germany.

9. German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany.

10. Behavioral and Epidemiology Research Group, American Cancer Society, Atlanta, Georgia, USA.

11. Center for Public Health Genomics, University of Virginia, Charlottesville, Virginia, USA.

12. Division of Gastroenterology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA.

13. Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA.

14. Clinical and Translational Epidemiology Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA.

15. Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA.

16. Department of Epidemiology, Harvard T.H. Chan School of Public Health, Harvard University, Boston, Massachusetts, USA.

17. Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Harvard University, Boston, Massachusetts, USA.

18. University Medical Centre Hamburg-Eppendorf, University Cancer Centre Hamburg (UCCH), Hamburg, Germany.

19. Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany.

20. Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California, USA.

21. Prevention and Cancer Control, Cancer Care Ontario, Toronto, Ontario.

22. Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Victoria, Australia.

23. Cancer Epidemiology and Intelligence Division, Cancer Council Victoria, Melbourne, Victoria, Australia.

24. Department of Medicine, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai

Medical Center, Los Angeles, California, USA.

25. Department of Nutrition, Harvard T.H. Chan School of Public Health, Harvard University, Boston, Massachusetts, USA.

26. Nutrition and Metabolism Section, International Agency for Research on Cancer, World Health Organization, Lyon, France.

27. Department of Medicine, University Hospital Dresden, Technische Universität Dresden (TU Dresden), Dresden, Germany.

28. University of Hawaii Cancer Center, Honolulu, Hawaii, USA.

29. PanCuRx Translational Research Initiative, Ontario, Institute for Cancer Research, Toronto, Ontario, Canada.

30. Department of Family Medicine, University of Virginia, Charlottesville, Virginia, USA.

31. Department of Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden.

32. Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden.

33. Área de Medicina Preventiva y Salud Publica, Universidad de León, Spain.

34. Cancer Prevention and Control Program, Catalan Institute of Oncology-IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain.

35. CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain.

36. Department of Clinical Sciences, Faculty of Medicine, University of Barcelona, Barcelona, Spain.

37. Department of Epidemiology, University of Washington School of Public Health and Community Medicine, Seattle, Washington, USA.

38. Clinical Genetics Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York, USA.

39. Department of Medicine, Weill Cornell Medical College, New York, New York, USA.

40. Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK.

41. Department of Community Medicine and Epidemiology, Lady Davis Carmel Medical Center, Haifa, Israel.

42. Ruth and Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel.

43. Clalit National Cancer Control Center, Haifa, Israel.

44. Division of Research, Kaiser Permanente Northern California, Oakland, California, USA.

45. Department of General and Thoracic Surgery, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany.

46. Department of Cancer Epidemiology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida, USA.

47. Division of Epidemiology, Department of Internal Medicine, University of Utah, Salt Lake City, Utah, USA.

48. Division of Laboratory Genetics, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA.

49. Huntsman Cancer Institute and Department of Population Health Sciences, University of Utah, Salt Lake City, Utah, USA.

50. Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.

51. Memorial University of Newfoundland, Discipline of Genetics, St. John's, Canada.

52. Vanderbilt Genetics Institute, Vanderbilt University Medical Center, Nashville, Tennessee, USA.

53. Department of Biomedical Informatics, Vanderbilt University Medical Center, Nashville, Tennessee, USA.

54. Department of Medical Oncology, City of Hope National Medical Center, Duarte, California, USA.

CONFLICTS OF INTEREST:

The authors declare no potential conflicts of interest.

CORRESPONDING AUTHOR:
Nikhil K. Khankari, PhD, MPH
Division of Epidemiology
Vanderbilt University Medical Center
2525 West End Ave, Suite 600
Nashville, TN 37203
Email: nikhil.khankari@vumc.org

Keywords: polyunsaturated fatty acids, aspirin, colorectal cancer, Mendelian randomization

Running title: Polyunsaturated fatty acids and colorectal cancer risk

ABSTRACT (247/250 words)

Background: Results from epidemiologic studies examining polyunsaturated fatty acids (PUFAs) and colorectal cancer (CRC) risk are inconsistent. Mendelian randomization may strengthen causal inference from observational studies. Given their shared metabolic pathway, examining the combined effects of aspirin/NSAID use with PUFAs could help elucidate an association between PUFAs and CRC risk.

Methods: Information was leveraged from GWAS regarding PUFA-associated single nucleotide polymorphisms (SNPs) to create weighted genetic scores (wGSs) representing genetically-predicted circulating blood PUFAs for 11,016 non-Hispanic white CRC cases and 13,732 controls in the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO). Associations per standard deviation increase in the wGS were estimated using unconditional logistic regression. Interactions between PUFA wGSs and aspirin/NSAID use on CRC risk were also examined.

Results: Modest CRC risk reductions were observed per standard deviation increase in circulating linoleic acid (OR_{LA}=0.96; 95% CI=0.93-0.98; p=5.2x10^{-4}), α-linolenic acid (OR_{ALA}=0.95; 95% CI=0.92-0.97; p=5.4x10^{-5}); whereas modest increased risks were observed for arachidonic acid (OR_{AA}=1.06; 95% CI=1.03-1.08; p=3.3x10^{-5}), eicosapentaenoic (OR_{EPA}=1.04; 95% CI=1.01-1.07; p=2.5x10^{-3}), and docosapentaenoic acids (OR_{DPA}=1.03; 95% CI=1.01-1.06; p=1.2x10^{-2}. Each of these effects were stronger among aspirin/NSAID non-users in the stratified analyses.

Conclusions: Our study suggests that higher circulating shorter-chain PUFAs (i.e., LA and ALA) were associated with reduced CRC risk, whereas longer-chain PUFAs (i.e., AA, EPA, and DPA) were associated with an increased CRC risk.

Impact: The interaction of PUFAs with aspirin/NSAID use indicates a shared CRC inflammatory pathway. Future research should continue to improve PUFA genetic instruments to elucidate the independent effects of PUFAs on CRC.

MANUSCRIPT: 4,334 words

Main Tables: 3

Supplementary Tables: 5

INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide with an estimated 746,000 males and 614,000 females diagnosed in 2012.[1] Diet has been shown to play an important role in CRC development.[2,3] One nutrition-related inflammatory metabolite, prostaglandin E2 (PGE-2), is known to influence colorectal carcinogenesis[4] via promotion of tumor cell proliferation[5,6] and silencing of tumor suppressor and DNA repair genes.[7] PGE-2 is generated via metabolism of omega-6 polyunsaturated fatty acid (PUFA) arachidonic acid (AA) via the cyclooxygenase-2 (COX-2) enzyme[4] and is often overexpressed in CRC.[8,9] While omega-3 polyunsaturated fatty acids (PUFAs) are also metabolized by COX-2, they produce a different array of non-inflammatory eicosanoids which have not been implicated in carcinogenesis. Thus, PGE-2 levels may be competitively reduced by increasing levels of omega-3 PUFAs in the diet, which could be a potential strategy for CRC prevention.

Dietary intake of PUFAs have been studied in relation to CRC incidence; however, the results from epidemiologic investigations have been inconsistent.[10–12] One possible reason for these discrepancies in the epidemiologic literature may be related to error in accurately assessing dietary PUFA intake. For example, differential recall of dietary intake in case-control studies of CRC could lead to biased effect estimates. In cohort studies, repeated measurements would be ideal but are not feasible, and a pre-diagnostic measurement of PUFAs using an objective dietary biomarker may not accurately reflect dietary intake since the etiologically relevant period for CRC development is unclear. The observed inconsistencies could also be due to biases related to inappropriate confounding control, selection bias, or reverse causation. In addition to these methodologic considerations, it is important to consider aspirin and non-steroidal anti-inflammatory drug (NSAID) use in tandem with PUFAs given their shared

metabolic pathway via COX-2 and resulting PGE-2 production. A limited number of studies have examined the interaction between PUFAs and aspirin/NSAID use on CRC risk with inconsistent results.[13,14]

The goal of our study was to estimate potentially unbiased associations between genetically-predicted circulating PUFAs with CRC using the Mendelian randomization approach. The Mendelian randomization approach uses genetic variants as instrumental variables for an exposure, and given alleles are randomly assorted during conception (akin to a randomized trial), results from such analyses are less susceptible to confounding and other biases[15]. Our study was conducted among non-Hispanic whites using data from two large CRC consortia. Given the shared metabolism via COX-2, we further assessed the combined effects of genetically-predicted circulating PUFAs and aspirin/NSAID use on CRC risk.

METHODS

Study population

The current study leverages the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO) consortium and the Colon Cancer Family Registry (CCFR), a pooled dataset of 14 studies of CRC with a total of 11,018 cases and 13,735 controls, all European ancestry. Details regarding the characteristics of individual studies included in the consortium have been published.[16–18] Briefly, medical records, pathologic reports, or death certificates were used to confirm colorectal cancer cases. Genotyped SNPs that did not meet the following criteria were excluded: (1) call rate <98%; (2) lack of Hardy-Weinberg equilibrium in the controls ($p<1x10^{-4}$); or (3) low minor allele frequencies.[16] All imputed SNPs had an $R^2>0.3$. Additional details regarding genotyping are published elsewhere.[19] Our study used individual-

level and summary statistics data from GECCO to conduct primary and sensitivity analyses. Additionally, summary statistics were available from the ColoRectal Transdisciplinary Study (CORECT) consortium, a pooled dataset comprised of 17 studies with a total of 18,682 cases and 11,225 controls are included. Study-specific sample sizes and genotyping platforms are provided in **Supplementary Table 1**. All study participants provided written informed consent, and all studies included in the consortia were approved by their respective institutional review boards.

Instrumental variable selection

Single nucleotide polymorphisms (SNPs) identified from published omega-6 and omega-3 PUFA GWAS conducted among individuals of European ancestry [20,21] were used as the genetic instruments for this Mendelian randomization analysis. The previous GWAS were conducted among the same individuals as part of the Cohorts for Heart and Aging Research in Genomic Epidemiology (i.e., CHARGE) Consortium. They reported associations between SNPs and plasma levels of omega-6 and omega-3 PUFAs (i.e., as a percentage of total fatty acids). The following nine SNPs were selected as they were all genome-wide significant (i.e., $p<5x10^{-8}$) and independent at $r^2<0.1$: rs10740118, rs174547, rs2727270, rs16966952, rs3798713, rs174538, rs780094, rs3734398, and rs2236212. The SNPs used in the six different genetic instruments (one instrument per PUFA) are summarized in **Table 1**, and further details are provided in **Supplementary Table 2**. Using the β estimates and effect allele frequencies (EAFs) specific to each SNP i, and the variance in PUFA levels from published GWAS [20,21], the percent variation explained by the n SNPs included in the six different genetic instruments were calculated as follows: $\sum_i^n [2\beta_i{}^2\ (MAF)(1-MAF)/variance(PUFA)] * 100$ [22]. In GECCO, the average imputation quality for imputed SNPs was $r^2=0.98$ (range: 0.97-0.99). In CORECT,

the average imputation quality was r^2=0.99 (range: 0.98-0.99).

Construction of weighted genetic scores

Weighted genetic scores (wGSs) were created using individual-level genotyped data in GECCO. For each PUFA, a wGS was constructed per individual as follows: $wGS = \sum_i^n \beta_i * dosage_i$; where n is the number of independent SNPs used for each PUFA instrument, β_i is the effect estimate (i.e., increase in percent of total plasma fatty acids) for SNP i (obtained from two GWAS examining omega-3 and omega-6 PUFAs within the same population [20,21]), and $dosage_i$ (range from 0-2) is the number of the effect alleles (i.e., alleles representing increased fatty acids levels) an individual possesses for SNP i. All GECCO participants had six different PUFA wGSs representing genetically-predicted circulating PUFA levels measured as a percentage of total plasma fatty acids. Excluding DHA's correlation with LA, AA, and ALA, the PUFA wGSs were highly correlated (**Supplementary Table 3)**. No wGSs were simultaneously included in a single model.

Statistical analysis

Unconditional logistic regression adjusted for age, sex, study, and top three principal components for European ancestry was conducted to estimate associations between one standard deviation increase in genetically-predicted circulating PUFAs and CRC risk in GECCO. Matching factors including age, sex, and study were included in the models to avoid any bias due to control selection.[23] We also adjusted for principal components of European ancestry to account bias due to population stratification.[24,25] We also explored the association between each PUFA wGS with potential confounders including education (highest level completed),

family history (first-degree relative), regular aspirin/NSAID use (at any point during a participant's lifetime), body mass index (BMI; kg/m^2), ever smoking (yes/no), alcohol use (g/day; compared to non-drinkers), folate intake (μg/day from diet), red meat consumption (serving/day), fruit and vegetable intake (servings/day), and sedentary behavior (hours/week; **Supplementary Table 4**). Only education, family history, aspirin/NSAID use, BMI, and fruit intake were found to be significantly associated ($p<0.05$) with the six different PUFA wGSs. Results from the fully-adjusted model adjusting for these covariates were identical to those from the minimally-adjusted models.

Analyses were stratified by potential effect measure modifiers including sex, age [i.e., <65 years (median age), ≥65 years], smoking use, regular aspirin/NSAID use, and BMI (i.e., ≤18.5 kg/m^2, 18.5-24.9, 25-30, and >30). Statistically significant differences ($p<0.05$) in strata were assessed via the likelihood ratio test using nested models for the multiplicative interaction term. Polytomous regression was conducted to estimate stratum-specific estimates by cancer site (i.e., rectal vs. colon, and separately for proximal and distal colon cancer).

Additive interactions were also conducted to assess the combined effects of genetically-predicted circulating PUFA levels and aspirin/NSAID use on CRC risk. All six PUFA-specific wGSs were dichotomized at the median representing "low" and "high" circulating levels. Using a common referent category, additive interactions were assessed statistically via calculation of the relative excess risk due to interaction (RERI) and its corresponding 95% confidence intervals.[26] All analyses were conducted using SAS Enterprise 7.13 (Cary, NC, USA) and "TwoSampleMR" package curated by MR-Base [27] in R 3.5.1 (R Foundation for Statistical Computing; https://www.r-project.org/).

Sensitivity analyses

Several sensitivity analyses were conducted in GECCO and CORECT. A fixed-effects inverse-variance weighted Mendelian randomization analysis[28] was conducted using summary statistics from PUFA GWAS and from the two consortia, GECCO and CORECT. The remaining analyses assessed the validity of the genetic instruments utilized in this study. Egger regression estimated a bias-reduced Mendelian randomization association in the presence of directional pleiotropy (i.e., when the average pleiotropic effects of all SNPs used in the instrument are either positive or negative), provided the effects of the instrument on the exposure is not correlated with any pleiotropic effects. Statistically significant intercepts from Egger regression indicate directional pleiotropy and was applied when three or more independent SNPs were included in the instrument (LA and DPA).[29] The weighted-median approach estimated the Mendelian randomization effect assuming at least 50% of SNPs used in the genetic instrument are invalid.[30] Corresponding 95% confidence intervals for the weighted-median estimate were calculated using bootstrapped standard errors. The weighted-median estimate was only conducted for the PUFAs with more than two SNPs in the instrument, and was not conducted for AA, ALA, DPA, or DHA. The multivariable Mendelian randomization was adjusted for the potential pleiotropic effects of the SNPs included in one PUFA instrument on circulating levels of other PUFAs and utilized all nine GWAS-identified SNPs and their PUFA-specific beta estimates.[31,32] Finally, for instruments with more than two SNPs, a "leave-one-out" analysis was conducted where the inverse-variance MR association was re-estimated after excluding the most influential SNP (determined via largest magnitude change in MR estimate after exclusion).[27] All sensitivity analyses using summary statistics were scaled to represent one standard deviation increase in genetically-predicted circulating PUFA levels.

RESULTS

The variants used in the six different PUFA genetic instruments are listed in **Table 1**. The instruments for α-linolenic acid (ALA) and docosahexaenoic acid (DHA) included one SNP each explaining 1.0% (i.e., rs174547) and 0.7% (i.e., rs2236212) percent of variation in PUFA levels, respectively. The instruments for eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA) explained a higher proportion of variance in fatty acid levels with 2.1% and 11.6%, respectively. Comparatively, the SNPs associated with omega-6 PUFAs, linoleic acid (LA) and arachidonic acid (AA), explained a higher percent variation in fatty acid levels. Four SNPs were significantly associated with and explained anywhere between 8.8 to 23.6% of the variation in circulating LA levels (reported range from studies included in the omega-6 GWAS [20]). For AA, two SNPs (i.e., rs174547 and rs16966952) together explained more than 33% of variation in AA fatty acid levels, with rs174547 accounting for most of the variation explained.

Main effects and stratified analyses

In **Table 2**, a one standard deviation increase in wGSs for shorter-chain omega-6 and omega-3 fatty acids (i.e., LA and ALA) was associated with 4% to 5% reduced CRC risk (OR_{LA}=0.96, 95% CI=0.93-0.98, p=5.2x10^{-4}; OR_{ALA}=0.95, 95% CI=0.92-0.97, p=5.4x10^{-5}). An increased CRC risk was observed per standard deviation increase in circulating longer-chain omega-3 fatty acids, EPA (OR_{EPA}=1.04, 95% CI=1.01-1.07, p=2.5x10^{-3}) and DPA (OR_{DPA}=1.03, 95% CI=1.01-1.06, p=1.2x10^{-2}). No association was observed for DHA. The largest observed increased risk was for AA, the longer-chain omega-6 PUFA, where a 6% increased CRC risk was observed (OR_{AA}=1.06, 95% CI=1.03-1.08, p=3.3x10^{-5}).

Stratified analyses are also presented in **Table 2**. Overall, most associations showed little evidence for varying by strata of different effect measure modifiers. Potential exceptions included a statistically significant multiplicative interaction for age (<65 years vs. ≥65 years; $p_{\text{interaction for LA}}=1.5\text{x}10^{-2}$ and $p_{\text{interaction for ALA}}=0.04$) and regular aspirin/NSAID use ($p_{\text{interaction for AA}}=0.05$, $p_{\text{interaction for ALA}}=0.04$, and $p_{\text{interaction for EPA}}=1.4\text{x}10^{-2}$). Among those ≥65 years, one standard deviation increase in genetically-predicted circulating ALA and LA reduced CRC risk by 7% and 8%, respectively ($OR_{\text{LA, ≥65 years}}=0.93$, 95% CI=0.89-0.96, $p=5.4\text{x}10^{-5}$; $OR_{\text{ALA, ≥65 years}}=0.92$, 95% CI=0.89-0.96, $p=2.7\text{x}10^{-5}$). Whereas among individuals <65 years, no statistically significant associations were observed. For longer-chain omega-3 PUFAs (i.e., EPA, DPA, and DHA), no differences across the age-stratified results were observed. For the longer-chain omega-6, one standard deviation increase in circulating AA levels was associated with an 8% increased CRC risk among those ≥65 years ($OR_{\text{AA, ≥65 years}}=1.08$, 95% CI=1.04-1.12, $p=2.7\text{x}10^{-5}$), and no association was observed among those <65 years ($OR_{\text{AA, <65 years}}=1.03$, 95% CI=0.99-1.07, p=0.08). Among aspirin/NSAIDs non-users, a similar 8% increased risk was observed per standard deviation increase in circulating AA ($OR_{\text{AA, aspirin/NSAID non-user}}=1.08$, 95% CI=1.04-1.11, $p=8.3\text{x}10^{-6}$), whereas no association was observed ($OR_{\text{AA, aspirin/NSAID user}}=1.02$, 95% CI=0.98-1.07, p=0.34) among users. For the short-chain omega-3 PUFA ALA, those individuals who were aspirin/NSAID non-users were observed to have a 7% reduced CRC risk per one standard deviation increase in circulating ALA levels ($OR_{\text{ALA, aspirin/NSAID non-user}}=0.93$, 95% CI=0.90-0.96, $p=9.7\text{x}10^{-6}$). Similar to longer-chain omega-6 AA, increased CRC risks were observed for higher levels of circulating longer-chain omega-3s EPA ($OR_{\text{EPA, aspirin/NSAID non-user}}=1.07$, 95% CI=1.03-1.10, $p=1.7\text{x}10^{-4}$) and DPA ($OR_{\text{DPA, aspirin/NSAID non-user}}=1.05$, 95% CI=1.02-1.09, $p=2.4\text{x}10^{-3}$) among aspirin/NSAID non-users; however this multiplicative

interaction was only statistically significant for EPA. Whereas among regular aspirin/NSAID users, null associations were observed for PUFAs in the stratified analysis.

Additive interaction with aspirin/NSAID use

In **Table 3**, additive interaction between PUFA-specific wGSs and regular use of aspirin/NSAID via a common referent category (i.e., "low" circulating PUFA levels and aspirin/NSAID non-users) are presented. Among those who were not regular aspirin/NSAID users (i.e., aspirin/NSAID non-users), high levels of circulating shorter-chain PUFAs (i.e., omega-6 LA and omega-3 ALA) was associated with an 11-13% reduction in CRC risk ($OR_{high\ LA,\ aspirin/NSAID\ non\text{-}user}$=0.89, 95% CI=0.84-0.95, $p=7.8x10^{-4}$; $OR_{high\ ALA,\ aspirin/NSAID\ non\text{-}user}$=0.87, 95% CI=0.81-0.93, $p=4.1x10^{-5}$). A 15% increased CRC risk was observed for higher levels of genetically-predicted circulating longer-chain omega-6 AA among aspirin/NSAID non-users ($OR_{AA,\ aspirin/NSAID\ non\text{-}user}$=1.15, 95% CI=1.07-1.23, $p=4.4x10^{-5}$). Similar increased CRC risks were observed for higher circulating levels of longer-chain omega-3 PUFAs EPA ($OR_{EPA,\ aspirin/NSAID\ non\text{-}user}$=1.12, 95% CI=1.05-1.20, $p=7.6x10^{-4}$) and DPA ($OR_{DPA,\ aspirin/NSAID\ non\text{-}user}$=1.07, 95% CI=1.00-1.15, $p=3.9x10^{-2}$), among aspirin/NSAID non-users.

Among those with lower levels of genetically-predicted circulating PUFAs, use of aspirin/NSAIDs was associated with reduced CRC risk, with CRC risk reductions ranging from 24% ($OR_{low\ AA,\ aspirin/NSAID\ user}$=0.76, 95% CI=0.70-0.82, $p=8.4x10^{-12}$) to 29% ($OR_{low\ LA,\ aspirin/NSAID\ user}$=0.71, 95% CI=0.65-0.77, $p=3.3x10^{-17}$). Generally, among aspirin/NSAID users, higher levels of genetically-predicted PUFAs (namely LA and ALA) did not further reduce CRC risk compared to lower levels of PUFAs ($OR_{high\ LA,\ aspirin/NSAID\ user}$=0.68, 95% CI=0.63-0.73, $p=2.0x10^{-20}$; $OR_{high\ ALA,\ aspirin/NSAID\ user}$=0.65, 95% CI=0.65, 95% CI=0.60-0.71, $p=3.2x10^{-25}$). For

longer-chain PUFAs (i.e., omega-6: AA, and omega-3s: EPA, DPA, and DHA), among aspirin/NSAID users, the effect of higher circulating levels of these PUFAs modestly attenuated the CRC risk reductions observed compared to lower levels of AA, EPA, DPA, and DHA. However, the additive interactions presented did not significantly deviate from an additive model as measured via the RERI and corresponding 95% CIs. Overall, CRC risk reductions (likely driven by aspirin/NSAID use) were still observed in this subgroup ($OR_{high\ AA,\ aspirin/NSAID\ user}$=0.82, 95% CI=0.76-0.89, $p=1.9x10^{-6}$; $OR_{high\ EPA,\ aspirin/NSAID\ user}$=0.80, 95% CI=0.74-0.87, $p=4.4x10^{-8}$; $OR_{high\ DPA,\ aspirin/NSAID\ user}$=0.77, 95% CI=0.71-0.83, $p=8.1x10^{-11}$; $OR_{high\ DHA,\ aspirin/NSAID\ user}$=0.80, 95% CI=0.73-0.87, $p=2.5x10^{-7}$).

Summary statistics and sensitivity analyses results

The inverse-variance weighted fixed-effects Mendelian randomization results (**Supplementary Table 5**) using summary statistics were identical to those from the individual-level wGS results. For PUFAs with more than one SNP included in the instrument, statistically significant heterogeneity was observed for the inverse-variance weighted fixed-effects MR estimates for DPA ($p_{heterogeneity}=3.6x10^{-4}$), indicating possibility for directional pleiotropy (i.e., when the effect on the outcome for each SNP included in the instrument is in the same direction).[15] The results in CORECT were identical to GECCO. Results from the weighted-median analyses were identical to the inverse-variance weighted fixed-effects MR, indicating that our estimates are robust when assuming half the variants included in the instrument are invalid.[30] No estimates from the multivariable MR approaches were statistically significant, which evaluated potential pleiotropy of SNPs included in one instrument on other PUFAs.[31,32] Results from the "leave-one-out" analysis (only possible for LA and DPA)

indicated that rs174547 was the most influential SNP in these two instruments, and removal of rs174547 from the PUFA instruments did not affect the overall results. The one exception being for DPA in the CORECT consortium where removal of rs174547 resulted in a 7% reduced CRC risk (OR_{DPA}=0.93, 95% CI=0.88-0.97, p=2.1x10^{-3}).

DISCUSSION

In our study conducted among over 24,000 non-Hispanic white individuals from the GECCO consortium, we observed a 6% increased CRC risk among those with higher genetically-predicted circulating levels of omega-6 PUFA AA. Modest increased risks were observed for EPA and DPA. Modest risk reductions were observed for longer-chain omega-6 PUFA LA, and longer-chain omega-3 PUFAs ALA. These associations remained statistically significant among those ≥65 years and among aspirin/NSAID non-users. When stratified by aspirin/NSAID use, one standard deviation increase in circulating AA increased risk of CRC by 8% ($p_{interaction}$=0.05), and reduced risk by 7% for ALA ($p_{interaction}$=0.04). Regular users of aspirin/NSAIDs were observed to have 18-35% reduced risk of CRC regardless of their genetically-predicted levels of PUFAs. Our main effects results were confirmed using the summary statistics Mendelian randomization approach.

Not all the associations observed were consistent with our biologic hypothesis regarding omega-6 and omega-3 PUFAs. For example, a modest 4% reduction in CRC risk was observed for increases in genetically-predicted short-chain omega-6 LA levels, which is a pre-cursor to AA levels and subsequently PGE-2. One potential explanation for the risk reduction observed for the LA may be related to two variants included in the instrument that are part of the *FADS1* and *FADS2* genes (i.e., rs174547 and rs2727270, respectively) and are responsible for the conversion

of LA to AA. When incorporating these SNPs in the instrument, increased genetically-predicted levels of LA will result in lower downstream levels of AA and PGE-2, which could potentially reduce CRC risk. We also observed modest increased risks for higher genetically-predicted levels of potentially anti-inflammatory omega-3 PUFAs EPA and DPA. However, the risk reduction is consistent with a previous meta-analysis of LA intake on CRC risk[33], and with a previous Mendelian randomization study (also included data from the CCFR) conducted by May-Wilson et al. among 7 European cohorts (OR_{LA}=0.95, 95% CI= 0.93-0.98).[34] Furthermore, results for AA from May-Wilson et al. (OR_{AA}=1.05, 95% CI=1.02-1.07) are nearly identical to those presented in our study. Results for EPA, DPA, and DHA were in the same direction (except for EPA); however, the effect sizes reported in May-Wilson et al. have larger magnitudes but are less precise. We also observed slightly stronger associations among older (i.e., ≥65 years) compared to younger individuals for many of the PUFAs, which could be an indication of the cumulative effects of being genetically-predisposed to higher PUFA levels on CRC risk.

The benefits of taking aspirin/NSAID on CRC risk has been studied extensively.[35,36] GECCO has also reported risk reductions with aspirin/NSAID use (OR=0.71, 95% CI=0.66-0.77),[37] and the magnitude of the risk reduction was similar to the associations reported among the subgroup of aspirin/NSAID users when considering the interactions with circulating PUFAs. Notably, in the Nurses' Health Study, long-term aspirin use (i.e., >10 years) and NSAID use reduced CRC risk by 32%, and risk was reduced by over 50% (OR=0.47, 95% CI=0.31-0.71) among women taking more than 14 (325-mg) tablets per week.[35] The benefits of long-term aspirin use were corroborated in randomized and observational studies.[36] The recommendation to the United States Preventive Task Force for long-term aspirin use as a

preventive strategy for CRC was indicated for 10 years post-initiation.[38] In our study, aspirin/NSAID use was defined as regular use over an individual's lifetime and this definition varied according to study. Thus, it is possible that heterogeneity in assessment of aspirin intake may affect the association between long-term aspirin use and CRC risk in our study; however, the associations observed are consistent with previous investigations.

Hall and colleagues examined the interaction between PUFA levels and aspirin use on CRC risk among men in the Physicians' Health Study.[14] They reported reduced CRC risk with higher intake of long-chain omega-3 PUFAs (i.e., Quartile 4 vs. Quartile 1, $OR_{Q4vs.Q1}$=0.34, 95% CI=0.15-0.82) among non-aspirin users. Similar to our results, the potential added benefit of increasing long-chain omega-3 intake among aspirin users was minimal when compared to non-aspirin users with low omega-3 intake.[14] Among the Nurses' Health Study and Health Professionals Follow-up Study participants, the potential modification of marine omega-3 dietary intake by aspirin/NSAID use on CRC risk was evaluated but no significant heterogeneity was reported.[13] Another study examined pre-diagnostic levels of the urinary PGE-2 metabolite (PGE-M) on colorectal adenoma risk stratified by aspirin use (>2 tablets per week) in the Nurses' Health Study.[39] Aspirin use was only beneficial among individuals with high levels of PGE-M. Arachidonic acid uptake by COX-2 is reduced in the presence of NSAIDs in colon cancer cells.[40] Similarly, reduced binding of DHA to COX-2 was observed when combined with a selective COX-2 inhibitor celecoxib.[41] Inhibition of PUFA metabolism via the COX-2 enzyme in the presence of aspirin may help to explain the potential antagonism observed for the interaction between PUFAs and aspirin on CRC risk.

Our study has several strengths. First, we utilized data from two large consortia of approximately 25,000 and 30,000 subjects (for GECCO and CORECT, respectively) to estimate

potentially unbiased association between PUFAs and CRC risk using the Mendelian randomization approach. The availability of individual-level GECCO data and several covariates was helpful for assessing the association between the PUFA-specific wGSs with CRC risk factors. This is one way to assess the validity of the genetic instrument in a Mendelian randomization analysis (i.e., the instrument should not be associated with confounders of the exposure-disease association).[15] We adjusted for additional covariates that were found to be associated with the six different PUFA wGS; however, the results from the adjusted models were identical to the minimally-adjusted models. We also conducted stratified analyses to estimate the association between genetically-predicted PUFAs among different subgroups. Several Mendelian randomization sensitivity analyses were conducted to assess the robustness of the results in the presence of pleiotropy, but these analyses are likely underpowered due to the limited number of independent SNPs included. Finally, we are one of the few studies to assess the additive interaction between genetically-predicted circulating PUFAs along with aspirin/NSAID use on CRC risk.

While our study has many strengths, there are several opportunities for improvement in future investigations. There was indication of directional pleiotropy in the Mendelian randomization sensitivity analyses (for DPA), and for some of the PUFAs, we were unable to estimate an effect for sensitivity analyses using summary statistics (i.e., Egger regression, weighted-median approach, leave-one-out analysis) due to the limited number of SNPs used in the genetic instrument. Several of the wGSs were highly correlated with one another in the individual-level analysis, which would affect the estimation of independent PUFA effects. However, incorporating additional SNPs as part of the genetic instrument in the future will increase the percent variation explained and subsequently increase the strength of the genetic

instrument. Stronger genetic instruments will ultimately help to further elucidate independent PUFA effects and provide a better opportunity to assess influence of pleiotropy on the Mendelian randomization estimates. Furthermore, using new weights from future GWAS that examine associations with longer-term PUFA biomarkers (e.g., adipose tissue and red blood cell) will help to clarify the potential causal role of PUFAs on CRC risk. The power to detect an OR at least 1.05 at an α=0.05 in our study ranged from approximately 5% (for DHA) to 62% (for AA), and is dependent upon the strength of the instrument.[42] Further, increasing the percent variation explained may allow for the detection of even smaller effects due to increased power. The associations derived from a Mendelian randomization analysis could help to identify the presence of a potential causal association between exposure and outcome. Many comparisons were made in this analysis and thus the potential for false-positive associations exists. However, most associations in our analysis remain statistically significant even after Bonferroni correction for multiple comparisons. Furthermore, our genetic instruments utilized SNPs previously reported to be associated with circulating PUFAs that have previously shown to have influence on carcinogenesis in experimental studies, and thus the analyses undertaken in this paper are based on an *a priori* biologic hypothesis. Finally, it would be worthwhile to conduct similar analyses in different populations to better understand the influence of PUFAs on CRC risk in populations where the ratio of omega-6 to omega-3 PUFAs may differ (e.g., Asians), and among populations where CRC risk is high (e.g., African Americans). Future investigations should consider identifying additional genetic variants associated with PUFA levels among different races which would facilitate conducting Mendelian randomization analyses in these populations.

Due to substantial amount of missing data for continuous measures of aspirin/NSAID use, we were unable examine the interaction between long-term aspirin/NSAID use and

circulating PUFAs on CRC risk. However, since selective COX-2 inhibitors may increase risk of cardiovascular disease with long-term use,[43] examining the potential added benefit of omega-3 PUFA intake with long-term use of selective COX-2 inhibitors may be futile realistically (unless among high-risk population subgroups). Finally, it is possible that the results from the additive interaction are subject to residual confounding given aspirin/NSAID use was self-reported.[44] Thus, future investigations with better long-term measures of aspirin/NSAID use should further examine the interaction with PUFAs, and also consider other potential biologic pathways.

In conclusion, we observed a 6% increased risk for CRC among those with higher genetically-predicted circulating levels of omega-6 PUFA AA, and similarly modest increased risks for longer-chain omega-3 PUFAs EPA and DPA. Risk reductions were observed among those with higher genetically-predicted circulating levels of short-chain omega-6 PUFA LA, and short-chain omega-3 PUFA ALA. Our study results indicate that among aspirin/NSAID users, the potential benefit of increasing long-chain omega-3 PUFAs may be minimal in terms of further reducing CRC risk. Results from the Mendelian randomization analysis using summary statistics corroborate our main effect findings. However, due to the limited number of variants used in some genetic instruments, an assessment of the influence of pleiotropy on our estimates could not be evaluated for all PUFAs. Given the small effects observed and the limited number of SNPs used in our genetic instruments, the clinical significance of our results is limited, and our results may only indicate a shared CRC inflammatory pathway for PUFAs and aspirin/NSAID use. Future Mendelian randomization studies should continue to improve the genetic instruments used which will help to further elucidate the effects of specific PUFAs on CRC risk.

FUNDING

N.K. Khankari is supported by National Institutes of Health NCI K99 CA215360. M.C. Borges is supported by a Skills Development Fellowship from the UK Medical Research Council (Grant number MR/P014054/1). P.C. Haycock is supported by CRUK Population Research Postdoctoral Fellowship C52724/A20138. M. Song is supported by the American Cancer Society (Grant number MRSG-17-220-01 – NEC), and the US NIH grants (K99 CA215314, R00 CA215314). The following funding information is for GECCO:

ASTERISK: a Hospital Clinical Research Program (PHRC-BRD09/C) from the University Hospital Center of Nantes (CHU de Nantes) and supported by the Regional Council of Pays de la Loire, the Groupement des Entreprises Françaises dans la Lutte contre le Cancer (GEFLUC), the Association Anne de Bretagne Génétique and the Ligue Régionale Contre le Cancer (LRCC).

COLO2&3: National Institutes of Health (R01 CA60987).

The Colon Cancer Family Registry (CFR) Illumina GWAS was supported by funding from the National Cancer Institute, National Institutes of Health (grant numbers U01 CA122839, R01 CA143247 to G Casey). The Colon CFR/CORECT Affymetrix Axiom GWAS and OncoArray GWAS were supported by funding from National Cancer Institute, National Institutes of Health (grant number U19 CA148107 to S Gruber). The Colon CFR participant recruitment and collection of data and biospecimens used in this study were supported by the National Cancer Institute, National Institutes of Health (grant number U01 CA167551) and through cooperative agreements with the following Colon CFR centers: Australasian Colorectal Cancer Family Registry (NCI/NIH grant numbers U01 CA074778 and U01/U24 CA097735), USC Consortium Colorectal Cancer Family Registry (NCI/NIH grant numbers U01/U24 CA074799), Mayo Clinic Cooperative Family Registry for Colon Cancer Studies (NCI/NIH grant number U01/U24

CA074800), Ontario Familial Colorectal Cancer Registry (NCI/NIH grant number U01/U24 CA074783), Seattle Colorectal Cancer Family Registry (NCI/NIH grant number U01/U24 CA074794), and University of Hawaii Colorectal Cancer Family Registry (NCI/NIH grant number U01/U24 CA074806), Additional support for case ascertainment was provided from the Surveillance, Epidemiology and End Results (SEER) Program of the National Cancer Institute to Fred Hutchinson Cancer Research Center (Control Nos. N01-CN-67009 and N01-PC-35142, and Contract No. HHSN2612013000121), the Hawai‘i Department of Health (Control Nos. N01-PC-67001 and N01-PC-35137, and Contract No. HHSN26120100037C, and the California Department of Public Health (contracts HHSN261201000035C awarded to the University of Southern California, and the following state cancer registries: AZ, CO, MN, NC, NH, and by the Victoria Cancer Registry and Ontario Cancer Registry.

DACHS: This work was supported by the German Research Council (BR 1704/6-1, BR 1704/6-3, BR 1704/6-4, CH 117/1-1, HO 5117/2-1, HE 5998/2-1, KL 2354/3-1, RO 2270/8-1 and BR 1704/17-1), the Interdisciplinary Research Program of the National Center for Tumor Diseases (NCT), Germany, and the German Federal Ministry of Education and Research (01KH0404, 01ER0814, 01ER0815, 01ER1505A and 01ER1505B).

DALS: National Institutes of Health (R01 CA48998 to M. L. Slattery).

Harvard cohorts (HPFS, NHS, PHS): HPFS is supported by the National Institutes of Health (P01 CA055075, UM1 CA167552, U01 CA167552, R01 CA137178, R01 CA151993, R35 CA197735, K07 CA190673, and P50 CA127003), NHS by the National Institutes of Health (R01 CA137178, P01 CA087969, UM1 CA186107, R01 CA151993, R35 CA197735, K07 CA190673, and P50 CA127003) and PHS by the National Institutes of Health (R01 CA042182).

Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO): National Cancer

Institute, National Institutes of Health, U.S. Department of Health and Human Services (U01 CA164930, U01 CA137088, R01 CA059045.This research was funded in part through the NIH/NCI Cancer Center Support Grant P30 CA015704.

MEC: National Institutes of Health (R37 CA54281, P01 CA033619, R01 CA063464, U01 CA164973). Also part of CORECT funding acknowledgements.

OFCCR: National Institutes of Health, through funding allocated to the Ontario Registry for Studies of Familial Colorectal Cancer (U01 CA074783); see CCFR section above. Additional funding toward genetic analyses of OFCCR includes the Ontario Research Fund, the Canadian Institutes of Health Research, and the Ontario Institute for Cancer Research, through generous support from the Ontario Ministry of Research and Innovation.

PLCO: Intramural Research Program of the Division of Cancer Epidemiology and Genetics and supported by contracts from the Division of Cancer Prevention, National Cancer Institute, NIH, DHHS. Funding was provided by National Institutes of Health (NIH), Genes, Environment and Health Initiative (GEI) Z01 CP 010200, NIH U01 HG004446, and NIH GEI U01 HG 004438.

PMH: National Institutes of Health (R01 CA076366 to P.A. Newcomb).

VITAL: National Institutes of Health (K05 CA154337).

WHI: The WHI program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, U.S. Department of Health and Human Services through contracts HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C, and HHSN271201100004C.

The following funding information is for studies included in CORECT:

ATBC: The ATBC Study was supported by the US Public Health Service contracts (N01-CN-45165, N01-RC-45035, N01-RC-37004, and HHSN261201000006C) from the National Cancer Institute.

ColoCare: This work was supported by the National Institutes of Health (grant numbers R01 CA189184 (Li/Ulrich), U01 CA206110 (Ulrich/Li/Siegel/Figueireido/Colditz, 2P30CA015704-40 (Gilliland), R01 CA207371 (Ulrich/Li)), the Matthias Lackas-Foundation, the German Consortium for Translational Cancer Research, and the EU TRANSCAN initiative.

Colorectal Cancer Transdisciplinary (CORECT) Study: The CORECT Study was supported by the National Cancer Institute, National Institutes of Health (NCI/NIH), U.S. Department of Health and Human Services (grant numbers U19 CA148107, R01 CA81488, P30 CA014089, R01 CA197350; P01 CA196569; R01 CA201407) and National Institutes of Environmental Health Sciences, National Institutes of Health (grant number T32 ES013678).

CPS-II: The American Cancer Society funds the creation, maintenance, and updating of the Cancer Prevention Study-II (CPS-II) cohort. This study was conducted with Institutional Review Board approval.

ESTHER/VERDI: This work was supported by grants from the Baden-Württemberg Ministry of Science, Research and Arts and the German Cancer Aid.

Kentucky: This work was supported by the following grant support: 1) Clinical Investigator Award from Damon Runyon Cancer Research Foundation (CI-8) and 2) NCI R01CA136726; and, we would like to acknowledge the staff at the Kentucky Cancer Registry

MCCS cohort recruitment was funded by VicHealth and Cancer Council Victoria. The MCCS was further supported by Australian NHMRC grants 509348, 209057, 251553 and 504711 and

by infrastructure provided by Cancer Council Victoria. Cases and their vital status were ascertained through the Victorian Cancer Registry (VCR) and the Australian Institute of Health and Welfare (AIHW), including the National Death Index and the Australian Cancer Database.

MECC: This work was supported by the National Institutes of Health, U.S. Department of Health and Human Services (R01 CA81488 to SBG and GR).

MSKCC: The work at Sloan Kettering in New York was supported by the Robert and Kate Niehaus Center for Inherited Cancer Genomics and the Romeo Milio Foundation. Moffitt: This work was supported by funding from the National Institutes of Health (grant numbers R01 CA189184, P30 CA076292), Florida Department of Health Bankhead-Coley Grant 09BN-13, and the University of South Florida Oehler Foundation. Moffitt contributions were supported in part by the Total Cancer Care Initiative, Collaborative Data Services Core, and Tissue Core at the H. Lee Moffitt Cancer Center & Research Institute, a National Cancer Institute-designated Comprehensive Cancer Center (grant number P30 CA076292).

NFCCR: This work was supported by an Interdisciplinary Health Research Team award from the Canadian Institutes of Health Research (CRT 43821); the National Institutes of Health, U.S. Department of Health and Human Serivces (U01 CA74783); and National Cancer Institute of Canada grants (18223 and 18226). The authors wish to acknowledge the contribution of Alexandre Belisle and the genotyping team of the McGill University and Génome Québec Innovation Centre, Montréal, Canada, for genotyping the Sequenom panel in the NFCCR samples. Funding was provided to Michael O. Woods by the Canadian Cancer Society Research Institute.

SEARCH: The University of Cambridge has received salary support in respect of PDPP from

the NHS in the East of England through the Clinical Academic Reserve. Cancer Research UK (C490/A16561); the UK National Institute for Health Research Biomedical Research Centres at the University of Cambridge.

SPAIN: The Spanish study was supported by Instituto de Salud Carlos III, co-funded by FEDER funds –a way to build Europe– (grants PI14-613 and PI09-1286), Agency for Management of University and Research Grants (AGAUR) of the Catalan Government (grant 2017SGR723), and Junta de Castilla y León (grant LE22A10-2). Sample collection of this work was supported by the Xarxa de Bancs de Tumors de Catalunya sponsored by Pla Director d'Oncología de Catalunya (XBTC), Plataforma Biobancos PT13/0010/0013 and ICOBIOBANC, sponsored by the Catalan Institute of Oncology.

The Swedish Low-risk Colorectal Cancer Study: The study was supported by grants from the Swedish research council; K2015-55X-22674-01-4, K2008-55X-20157-03-3, K2006-72X-20157-01-2 and the Stockholm County Council (ALF project).

Swedish Mammography Cohort and Cohort of Swedish Men: This work is supported by the Swedish Research Council /Infrastructure grant, the Swedish Cancer Foundation, and the Karolinska Institute´s Distinguished Professor Award to Alicja Wolk.

ACKNOWLEDGEMENTS

The following acknowledgements are for GECCO:

ASTERISK: We are very grateful to Dr. Bruno Buecher without whom this project would not have existed. We also thank all those who agreed to participate in this study, including the patients and the healthy control persons, as well as all the physicians, technicians and students.

DACHS: We thank all participants and cooperating clinicians, and Ute Handte-Daub, Utz

Benscheid, Muhabbet Celik and Ursula Eilber for excellent technical assistance.

Harvard cohorts: The study protocol was approved by the institutional review boards of the Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health, and those of participating registries as required. We would like to thank the participants and staff of the HPFS, NHS and PHS for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. The authors assume full responsibility for analyses and interpretation of these data.

PLCO: The authors thank the PLCO Cancer Screening Trial screening center investigators and the staff from Information Management Services Inc and Westat Inc. Most importantly, we thank the study participants for their contributions that made this study possible.

PMH: The authors would like to thank the study participants and staff of the Hormones and Colon Cancer study.

WHI: The authors thank the WHI investigators and staff for their dedication, and the study participants for making the program possible. A full listing of WHI investigators can be found at: http://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Short%20List.pdf

The following acknowledgements are for CORECT:

ColoCare: Biospecimens were provided by the ColoCare Consortium, funded by the Fred Hutchinson Cancer Research Center. Other investigators may have received specimens from the same subjects**.**

CPS-II: The authors thank the CPS-II participants and Study Management Group for their

invaluable contributions to this research. The authors would also like to acknowledge the contribution to this study from central cancer registries supported through the Centers for Disease Control and Prevention National Program of Cancer Registries, and cancer registries supported by the National Cancer Institute Surveillance Epidemiology and End Results program.

MCCS: This study was made possible by the contribution of many people, including the original investigators and the diligent team who recruited participants and continue to work on follow-up. We would also like to express our gratitude to the many thousands of Melbourne residents who took part in the study and provided blood samples.

SEARCH: We acknowledge the contributions of Mitul Shah, Val Rhenius, Sue Irvine, Craig Luccarini, Patricia Harrington, Don Conroy, Rebecca Mayes, and Caroline Baynes.

The Swedish low-risk colorectal cancer study: We thank Berith Wejderot and the Swedish low-risk colorectal cancer study group.

DISCLAIMER

Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer/World Health Organization.

REFERENCES

1 Torre LA, Bray F, Siegel RL, *et al.* Global cancer statistics, 2012. *CA Cancer J Clin* 2015;**65**:87–108. doi:10.3322/caac.21262

2 Lippi G, Mattiuzzi C, Cervellin G. Meat consumption and cancer risk: a critical review of published meta-analyses. *Crit Rev Oncol Hematol* 2016;**97**:1–14. doi:10.1016/j.critrevonc.2015.11.008

3 Theodoratou E, Timofeeva M, Li X, *et al.* Nature, Nurture, and Cancer Risks: Genetic and Nutritional Contributions to Cancer. *Annu Rev Nutr* 2017;**37**:293–320. doi:10.1146/annurev-nutr-071715-051004

4 Wang D, DuBois RN. An inflammatory mediator, prostaglandin E2, in colorectal cancer. *Cancer J Sudbury Mass* 2013;**19**:502–10. doi:10.1097/PPO.0000000000000003

5 Kawamori T. Enhancement of colon carcinogenesis by prostaglandin E2 administration. *Carcinogenesis* 2003;**24**:985–90. doi:10.1093/carcin/bgg033

6 Wang D, Wang H, Shi Q, *et al.* Prostaglandin E(2) promotes colorectal adenoma growth via transactivation of the nuclear peroxisome proliferator-activated receptor delta. *Cancer Cell* 2004;**6**:285–95. doi:10.1016/j.ccr.2004.08.011

7 Xia D, Wang D, Kim S-H, *et al.* Prostaglandin E2 promotes intestinal tumor growth via DNA methylation. *Nat Med* 2012;**18**:224–6. doi:10.1038/nm.2608

8 Eberhart CE, Coffey RJ, Radhika A, *et al.* Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* 1994;**107**:1183–8.

9 Zhang H, Sun X-F. Overexpression of cyclooxygenase-2 correlates with advanced stages of colorectal cancer. *Am J Gastroenterol* 2002;**97**:1037–41. doi:10.1111/j.1572-0241.2002.05625.x

10 Gerber M. Omega-3 fatty acids and cancers: a systematic update review of epidemiological studies. *Br J Nutr* 2012;**107**:S228–39. doi:10.1017/S0007114512001614

11 Shen X-J, Zhou J-D, Dong J-Y, *et al.* Dietary intake of n-3 fatty acids and colorectal cancer risk: a meta-analysis of data from 489 000 individuals. *Br J Nutr* 2012;**108**:1550–6. doi:10.1017/S0007114512003546

12 Sakai M, Kakutani S, Horikawa C, *et al.* Arachidonic acid and cancer risk: a systematic review of observational studies. *BMC Cancer* 2012;**12**:606-2407-12–606.

13 Song M, Chan AT, Fuchs CS, *et al.* Dietary intake of fish, ω-3 and ω-6 fatty acids and risk of colorectal cancer: A prospective study in U.S. men and women: Fish, ω-3 and ω-6 fatty acids and risk of CRC. *Int J Cancer* 2014;**135**:2413–23. doi:10.1002/ijc.28878

14 Hall MN, Campos H, Li H, *et al.* Blood Levels of Long-Chain Polyunsaturated Fatty Acids, Aspirin, and the Risk of Colorectal Cancer. *Cancer Epidemiol Biomarkers Prev* 2007;**16**:314–21. doi:10.1158/1055-9965.EPI-06-0346

15 Haycock PC, Burgess S, Wade KH, *et al.* Best (but oft-forgotten) practices: the design, analysis, and interpretation of Mendelian randomization studies. *Am J Clin Nutr* 2016;**103**:965–78. doi:10.3945/ajcn.115.118216

16 Peters U, Jiao S, Schumacher FR, *et al.* Identification of Genetic Susceptibility Loci for Colorectal Tumors in a Genome-Wide Meta-analysis. *Gastroenterology* 2013;**144**:799-807.e24. doi:10.1053/j.gastro.2012.12.020

17 Hutter CM, Chang-Claude J, Slattery ML, *et al.* Characterization of Gene-Environment Interactions for Colorectal Cancer Susceptibility Loci. *Cancer Res* 2012;**72**:2036–44. doi:10.1158/0008-5472.CAN-11-4067

18 Newcomb PA, Baron J, Cotterchio M, *et al.* Colon Cancer Family Registry: An International Resource for Studies of the Genetic Epidemiology of Colon Cancer. *Cancer Epidemiol Biomarkers Prev* 2007;**16**:2331–43. doi:10.1158/1055-9965.EPI-07-0648

19 Zanke BW, Greenwood CM, Rangrej J, *et al.* Genome-wide association scan identifies a colorectal cancer susceptibility locus on chromosome 8q24. *Nat Genet* 2007;**39**:989–94. doi:10.1038/ng2089

20 Guan W, Steffen BT, Lemaitre RN, *et al.* Genome-wide association study of plasma N6 polyunsaturated fatty acids within the cohorts for heart and aging research in genomic epidemiology consortium. *Circ Cardiovasc Genet* 2014;**7**:321–31. doi:10.1161/CIRCGENETICS.113.000208

21 Lemaitre RN, Tanaka T, Tang W, *et al.* Genetic loci associated with plasma phospholipid n-3 fatty acids: a meta-analysis of genome-wide association studies from the CHARGE Consortium. *PLoS Genet* 2011;**7**:e1002193. doi:10.1371/journal.pgen.1002193

22 Shim H, Chasman DI, Smith JD, *et al.* A Multivariate Genome-Wide Association Analysis of 10 LDL Subfractions, and Their Response to Statin Treatment, in 1868 Caucasians. *PLOS ONE* 2015;**10**:e0120758. doi:10.1371/journal.pone.0120758

23 Rothman KJ, Greenland S, Lash TL. *Modern epidemiology*. 3 , [thoroughly rev a updat. Philadelphia: : Wolters Kluwer Health/Lippincott Williams & Wilkins 2008. http://www.loc.gov/catdir/enhancements/fy0828/2007036316-t.html

24 Campbell CD, Ogburn EL, Lunetta KL, *et al.* Demonstrating stratification in a European American population. *Nat Genet* 2005;**37**:868–72. doi:10.1038/ng1607

25 Price AL, Patterson NJ, Plenge RM, *et al.* Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 2006;**38**:904–9. doi:10.1038/ng1847

26 Hosmer DW, Lemeshow S. Confidence interval estimation of interaction. *Epidemiol Camb Mass* 1992;**3**:452–6.

27 Hemani G, Zheng J, Elsworth B, *et al.* The MR-Base platform supports systematic causal inference across the human phenome. *eLife* 2018;**7**. doi:10.7554/eLife.34408

28 Burgess S, Butterworth A, Thompson SG. Mendelian Randomization Analysis With Multiple Genetic Variants Using Summarized Data: Mendelian Randomization Using Summarized Data. *Genet Epidemiol* 2013;**37**:658–65. doi:10.1002/gepi.21758

29 Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol* 2015;**44**:512–25. doi:10.1093/ije/dyv080

30 Bowden J, Davey Smith G, Haycock PC, *et al.* Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. *Genet Epidemiol* 2016;**40**:304–14. doi:10.1002/gepi.21965

31 Burgess S, Thompson SG. Multivariable Mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects. *Am J Epidemiol* 2015;**181**:251–60. doi:10.1093/aje/kwu283

32 Burgess S, Dudbridge F, Thompson SG. Re: "Multivariable Mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects." *Am J Epidemiol* 2015;**181**:290–1. doi:10.1093/aje/kwv017

33 Zock PL, Katan MB. Linoleic acid intake and cancer risk: a review and meta-analysis. *Am J Clin Nutr* 1998;**68**:142–53.

34 May-Wilson S, Sud A, Law PJ, *et al.* Pro-inflammatory fatty acid profile and colorectal cancer risk: A Mendelian randomisation analysis. *Eur J Cancer* 2017;**84**:228–38. doi:10.1016/j.ejca.2017.07.034

35 Chan AT, Giovannucci EL, Meyerhardt JA, *et al.* Long-term use of aspirin and nonsteroidal anti-inflammatory drugs and risk of colorectal cancer. *JAMA* 2005;**294**:914–23. doi:10.1001/jama.294.8.914

36 Flossmann E, Rothwell PM, British Doctors Aspirin Trial and the UK-TIA Aspirin Trial. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. *Lancet Lond Engl* 2007;**369**:1603–13. doi:10.1016/S0140-6736(07)60747-8

37 Nan H, Hutter CM, Lin Y, *et al.* Association of Aspirin and NSAID Use With Risk of Colorectal Cancer According to Genetic Variants. *JAMA* 2015;**313**:1133. doi:10.1001/jama.2015.1815

38 Chubak J, Whitlock EP, Williams SB, *et al.* Aspirin for the Prevention of Cancer Incidence and Mortality: Systematic Evidence Reviews for the U.S. Preventive Services Task Force.

Ann Intern Med 2016;**164**:814. doi:10.7326/M15-2117

39 Bezawada N, Song M, Wu K, *et al.* Urinary PGE-M Levels Are Associated with Risk of Colorectal Adenomas and Chemopreventive Response to Anti-Inflammatory Drugs. *Cancer Prev Res (Phila Pa)* 2014;**7**:758–65. doi:10.1158/1940-6207.CAPR-14-0120

40 Orido T, Fujino H, Kawashima T, *et al.* Decrease in uptake of arachidonic acid by indomethacin in LS174T human colon cancer cells; a novel cyclooxygenase-2-inhibition-independent effect. *Arch Biochem Biophys* 2010;**494**:78–85. doi:10.1016/j.abb.2009.11.025

41 Swamy MV, Cooma I, Patlolla JMR, *et al.* Modulation of cyclooxygenase-2 activities by the combined action of celecoxib and decosahexaenoic acid: novel strategies for colon cancer prevention and treatment. *Mol Cancer Ther* 2004;**3**:215–21.

42 Burgess S. Sample size and power calculations in Mendelian randomization with a single instrumental variable and a binary outcome. *Int J Epidemiol* 2014;**43**:922–9. doi:10.1093/ije/dyu005

43 Gunter BR, Butler KA, Wallace RL, *et al.* Non-steroidal anti-inflammatory drug-induced cardiovascular adverse events: a meta-analysis. *J Clin Pharm Ther* 2017;**42**:27–38. doi:10.1111/jcpt.12484

44 Knol MJ, VanderWeele TJ. Recommendations for presenting analyses of effect modification and interaction. *Int J Epidemiol* 2012;**41**:514–20. doi:10.1093/ije/dyr218